- OFE 14A FOR DEC O 6 2005 BELLO
 - 1. (currently amended) A method of assaying pyrrole-containing biological compounds in a sample, the method comprising:
 - i) contacting [[a]] the sample biological compound with one of:
 - an Ehrlich's reagent covalently attached to a solid support
 optionally labelled derivatizing agent (bound to or able to bind
 with a solid support), wherein the Ehrlich's reagent derivatizing
 agent forms a reaction product with the biological compound,
 followed by exposure of the reaction product to a first antibody
 detectable molecule which forms a complex with the reaction
 product; or
 - b) an optionally labelled derivatizing agent not bound to a solid support, wherein the derivatizing agent forms a reaction product with the biological compound, followed by exposure to a binding agent specific to the biological compound in the reaction product, said binding agent being bound to a solid support; or
 - e) a binding agent bound to a solid support, said binding agent
 being specific to the biological compound and forming a complex
 therewith, followed by exposure to an optionally labelled,
 derivatizing agent which forms a reaction product with the
 biological compound moiety of said complex, and
 - ii) determining the amount of bound biological compound by detecting the complex. detectable molecule, or by determining the amount of free or bound binding agent or by measuring the amount of label present; thereby assaying pyrrole containing biological compounds.

- 2. (currently amended) The method according to claim 1, wherein the Ehrlich's reagent has the following formula comprising:
 - (a) -- contacting the biological compound with a derivatizing agent of the following structure in the bound form:

wherein R¹ is an alkyl group, R² is an alkyl alkylene group, A is a linking group and B is a solid support, and wherein the contact induces formation of a reaction product, and wherein the reaction product comprises the covalent attachment of the biological compound to the derivatizing agent;

- (b) contacting the reaction product with a detectable molecule, wherein the contact induces specific binding of the detectable molecule to the reaction product to provide a complex;
- (c) detecting the detectable molecule; thereby assaying pyrrole containing biological compounds.
- 3. (currently amended) The method according to claim 2, wherein R¹ is a straight-chain alkyl group containing 1 to 10 carbon atoms, and R² is a straight-chain alkyl alkylene group containing 1 to 10 carbon atoms.
- 4. (currently amended) The method according to claim 2, wherein A is a heteroalkyl heteroalkylene group.

- 5. (currently amended) The method according to claim 1 [[2]], wherein the antibody detectable molecule is a first monoclonal antibody.
- 6. (currently amended) The method according to claim 3, wherein R¹ is a straight-chain alkyl group containing 1 to 5 carbon atoms and R² is a straight chain alkyl alkylene group containing 1 to 5 carbon atoms.
- 7. (currently amended) The method according to claim 4, wherein A is a heteroalkyl heteroalkylene group comprising at least one nitrogen atom.
- 8. (currently amended) The method according to claim 1 [[5]], wherein the method further comprises contacting the complex is detected with a second monoclonal antibody that specifically binds to the first monoclonal antibody.
- 9. (currently amended) The method according to claim 6, wherein the <u>Ehrlich's</u>

 <u>reagent derivatizing agent</u> is of the following structure in bound form:

- 10. (currently amended) The method according to claim 9, wherein A is a heteroalkyl heteroalkylene group containing at least one nitrogen atom, and wherein the detectable molecule is a first monoclonal antibody.
- 11. (cancelled)

- 12. (currently amended) A method of assaying pyrrole-containing biological compounds in a sample, the method The method according to claim 1 comprising:
 - a) contacting the <u>sample</u> biological compound with an <u>a biotinylated</u>

 Ehrlich's reagent optionally labelled derivatizing agent in solution to form a reaction product therewith, followed by exposure of the reaction product to a <u>first antibody binding agent</u> bound to a solid support wherein the <u>first antibody binding agent is specific to the biological compound in forms a complex with the reaction product; and</u>
 - b) determining the amount of bound biological compound by determining the complex amount of labelled dirivatizing agent bound to the solid support; thereby assaying the pyrrole-containing compound.
- 13. (cancelled)
- 14. <u>(currently amended)</u> The method according to claim 12 wherein the derivatizing agent Ehrlich's reagent is labelled labeled with a radiolabel, fluorescent label or an enzyme label.
- 15. (cancelled) The method according to claim 12 wherein the solution containing the reaction product is neutralized prior to contact with the binding agent.
- 16. (currently amended) A method of assaying pyrrole-containing biological compounds in a sample, the method The method according to claim 1:
 - a) contacting the <u>sample</u> biological compound with a <u>biotinylated</u>

 <u>Ehrlich's reagent</u> derivatizing agent in solution to form a reaction product, wherein the derivatizing agent comprises a first partner of a strong binding pair;

- b) contacting the reaction product with a solid support <u>coated with</u>

 <u>strepavidin or avidin having a second partner of the strong binding</u>

 <u>pair on its surface, to form a bound complex, wherein with the reaction</u>

 product <u>is bound to the solid support; and</u>
- c) contacting the bound complex with a detectable molecule;
- d)—determining the amount of bound biological compound; thereby assaying the pyrrole-containing biological compound.
- 17. (cancelled)
- 18. (currently amended) The method according to claim 16 wherein the detectable molecule is a monoclonal antibody specific to the biological compound and the amount of bound biological compound is determined by detecting the amount MAb bound to the solid support reaction product is further contacted with a first antibody that binds to the reaction product to form a complex, and the complex is determined by a second antibody that is labeled and binds to the first antibody.
- 19. (currently amended) A method as claimed in The method of claim 16, wherein the Ehrlich's reagent has comprising: a) contacting the biological compound with a derivatizing agent of the following structure: in the bound form;

wherein R¹ is an alkyl group, R² is an alkyl alkylene group, R⁴ is a heteroalkyl heteroalkylene group, X is biotinyl the first labelling molecule

and acts as a first partner of a strong binding pair and Y is streptavidin or avidin attached to a solid support the second labelling molecule and acts as a second partner of a strong binding pair on the surface of a solid support, and wherein the contact induces formation of a reaction product, and wherein the reaction product comprises the covalent attachment of the biological compound to the derivatizing agent; b) contacting the reaction product with a detectable molecule, wherein the contact induces specific binding of the detectable molecule to the reaction product to provide a complex; c) detecting the detectable molecule; thereby assaying pyrrole containing biological compounds.

- 20. (currently amended) The method according to claim 19, wherein R¹ is a straight-chain alkyl group containing 1 to 10 carbon atoms, and R² is a straight-chain alkyl alkylene group containing 1 to 10 carbon atoms.
- 21. (currently amended) The method according to claim 19, wherein R⁴ is a straight-chain heteroalkyl heteroalkylene group containing 2 to 10 carbon atoms and at least 2 heteroatoms.
- 22. (previously presented) The method according to claim 19, wherein Y is a solid support having avidin on its surface.
- 23. (currently amended) The method according to claim 18 19, wherein the detectable molecule is a first antibody is a monoclonal antibody.
- 24. (currently amended) The method according to claim 19, wherein R¹ is a straight-chain alkyl group containing 1 to 5 carbon atoms, and R² is a straight chain alkyl alkylene group containing 1 to 5 carbon atoms.

- 25. (currently amended) The method according to claim 18, wherein the method further comprises contacting the complex with a second antibody is a monoclonal antibody that specifically binds to the first monoclonal antibody.
- 26. (currently amended) The method according to claim <u>19</u> <u>25</u>, wherein the derivatizing agent, in bound form, is of the following structure:

$$R_1$$
 R_2 R_4 $(CH_2)_4$ S $NH:Y$

wherein R¹ is a straight-chain alkyl group containing 1 to 5 carbon atoms, R² is a straight-chain alkyl alkylene group containing 1 to 5 carbon atoms, R⁴ is a straight-chain heteroalkyl heteroalkylene group containing 2 to 10 carbon atoms and at least 2 heteroatoms, and Y is a solid support having avidin or streptavidin on its surface.

- 27. (previously presented) The method according to claim 26, wherein R^1 is $-CH_3$, R^2 is $-CH_2CH_2$ and R^4 is $-NH(CH_2)_5NH$ or -NH (CH₂) $_2SS$ (CH₂) $_2NHC$ (O)(CH₂) $_5NH$ —.
- 28. (previously presented) The method according to claim 27, wherein R^4 is $NH(CH_2)_5NH$ –.
- 29. (new) The method of claim 12, wherein the Ehrlich's reagent has the following structure:

$$R_1$$
 R_2 R_4 R_4

wherein R^1 is an alkyl group, R^2 is an alkylene group, R^4 is a heteroalkylene group, and X is biotinyl.

- 30. (new) The method according to claim 29, wherein R¹ is a straight-chain alkyl group containing 1 to 10 carbon atoms, and R² is a straight-chain alkylene group containing 1 to 10 carbon atoms.
- 31. (new) The method according to claim 29, wherein R⁴ is a straight-chain heteroalkylene group containing 2 to 10 carbon atoms and at least 2 heteroatoms.
- 33. (new) The method according to claim 12, wherein the first antibody is a monoclonal antibody.
- 34. (new) The method according to claim 29, wherein R¹ is a straight-chain alkyl group containing 1 to 5 carbon atoms, and R² is a straight chain alkylene group containing 1 to 5 carbon atoms.
- 35. (new) The method according to claim 12, wherein the second antibody is a monoclonal antibody.
- 36. (new) The method according to claim 29, wherein the derivatizing agent, is of the following structure:

$$R_1$$
 R_2 R_4 $(CH_2)_4$ S NH_2 NH_2

wherein R^1 is a straight-chain alkyl group containing 1 to 5 carbon atoms, R^2 is a straight-chain alkylene group containing 1 to 5 carbon atoms, and R^4 is a straight-chain heteroalkylene group containing 2 to 10 carbon atoms and at least 2 heteroatoms, and Y is a solid support having avidin or streptavidin on its surface.

- 37. (new) The method according to claim 36, wherein R^1 is $-CH_3$, R^2 is $-CH_2CH_2$ —and R^4 is $-NH(CH_2)_5NH$ —or -NH (CH_2) $_2SS$ (CH_2) $_2NHC$ (O)(CH_2) $_5NH$ —.
- 38. (new) The method according to claim 37, wherein R⁴ is -NH(CH₂)₅NH-.

JDE:KLI:elew (2672272)